Rule 1.26

What is claimed is:

1. A detector oligonucleotide, comprising at least two pairs of a donor fluorophore and a quencher molecule in close proximity, wherein said donor fluorophore and said quencher molecule in each said pair are separated by a cleavage site that is cleavable in a double-stranded form, and wherein cleavage at said cleavage site is capable of creating a detectable signal that indicates the presence of a target nucleic acid.

2. A single-stranded first detector oligonucleotide, comprising at least two pairs of a donor fluorophore and a quencher molecule in close proximity, wherein said donor fluorophore and said quencher molecule in each said pair are separated by a cleavage site that is cleavable in a double-stranded form, and wherein the cleavage site is double-stranded when the first detector oligonucleotide forms a duplex with a second oligonucleotide that is capable of being formed in the presence of a target nucleic acid.

Rule 1.26

3. The detector oligonucleotide of claim 2, wherein at least one said donor fluorophore is selected from the group consisting of fluorescein, sulforhodamine 101, pyrenebutanoate, acridine, ethenoadenosine, eosin, rhodamine, and erythrosine.

4. The oligonucleotide of claim 2, wherein at least one said quencher molecule is selected from the group consisting of DABCYL, DAMBI, DABSYL and methyl red.

5. The oligonucleotide of claim 2, wherein said donor fluorophores and said quencher molecules are separated by about 5 to 20 nucleic acid bases that comprise a

Rule 1.26

cleavage site.

6. The oligonucleotide of claim 5, wherein said donor fluorophores and said quencher molecules are separated by about 6 to 8 nucleic acid bases that comprise a cleavage site.

7. The oligonucleotide of claim 2, wherein said cleavage site is cleavable by a chemical cleavage reagent.

8. The oligonucleotide of claim 2, wherein said cleavage site is cleavable by an endonuclease.

9. The oligonucleotide of claim 8, wherein said endonuclease is selected from the group consisting of Hinc II, Nci I, and BsoB1.

Rule 1.26

10. The oligonucleotide of claim 2, comprising ten donor/quencher pairs.

11. The oligonucleotide of claim 10, comprising 50 donor/quencher pairs.

12. The oligonucleotide of claim 2, wherein the first detector oligonucleotide comprises a first portion at a 5' terminus that is capable of forming a duplex with a first portion at a 3' terminus of a second oligonucleotide, wherein the second oligonucleotide comprises a second portion that is complementary to a target nucleic acid and a third portion at a 5' terminus of the second oligonucleotide that comprises one strand of an endonuclease recognition site.

13. The oligonucleotide of claim 2, wherein said first detector oligonucleotide comprises a first portion capable of forming a duplex with a third oligonucleotide, wherein said third oligonucleotide comprises two portions: (1) a first portion having a

Rule 1.126

sequence capable of forming a duplex with a target nucleic acid and (2) a second portion capable of forming a duplex with said first portion of said first detector oligonucleotide.

14. The detector oligonucleotide of claim 13, where in said first portion is at the 5' terminus of said first detector oligonucleotide.

15. The oligonucleotide of claim 2, wherein said first detector oligonucleotide comprises a first portion capable of forming a duplex with a third oligonucleotide, wherein said third oligonucleotide comprises two portions: (1) a first portion having a sequence complementary to a target nucleic acid and (2) a second portion capable of forming a duplex with said first portion of said first detector oligonucleotide.

Rule 1.126

16. A partially double-stranded detector oligonucleotide, comprising at least two pairs of a donor fluorophore and a quencher molecule in close proximity, wherein said donor fluorophore and said quencher molecule in each said pair are separated by a cleavage site, wherein said partially double-stranded detector oligonucleotide comprises a single-stranded portion that is capable of forming a duplex with a target nucleic acid.

17. The oligonucleotide of claim 16, wherein at least one of said cleavage sites is cleavable by a chemical cleavage reagent.

18. The oligonucleotide of claim 16, wherein at least one of said cleavage sites is cleavable by an endonuclease.

19. A method for detecting a target nucleic acid, comprising:

a. contacting (i) a first detector oligonucleotide comprising a single-stranded portion that comprises at least two pairs of a donor

Rule 1.26

fluorophore and a quencher molecule in close proximity, wherein said donor fluorophore and said quencher molecule in each said pair are separated by a cleavage site, with (ii) a single-stranded second oligonucleotide, the presence of which is indicative of the presence of the target nucleic acid, to form a duplex between said first and second oligonucleotides;

b. extending the duplex to make said single-stranded portion of the first detector oligonucleotide double-stranded;

c. cleaving at least one of said cleavage sites; and

d. detecting said donor fluorophores,

Rule 1.26

wherein a detectable change in a fluorescence parameter of said donor fluorophores is indicative of the presence of said target nucleic acid.

20. The method of claim 18, wherein the first oligonucleotide comprises a first portion at a 5' terminus of the first oligonucleotide that forms a duplex with a first portion at a 3' terminus of the second oligonucleotide.

21. The method of claim 18, further comprising amplifying the second oligonucleotide.

22. The method of claim 21, wherein the second oligonucleotide is amplified before step (a).

23. The method of claim 21, wherein the second oligonucleotide comprises

Rule 1.126

one strand of an endonuclease recognition site in a third portion of the second oligonucleotide that is 5' of said second portion that is complementary to a target nucleic acid.

24. The method of claim 21, wherein the amplifying is by a method selected from the group consisting of strand displacement amplification (SDA), polymerase chain reaction (PCR), ligase chain reaction, self-sustained sequence replication (3SR), Q beta replicase-based amplification, solid phase amplification, nucleic acid sequence-based amplification (NASBA), rolling circle amplification, and transcription mediated amplification (TMA).

25. The method of claim 23, wherein the amplifying is by the method of strand displacement amplification.

Rule 1.126

26. The method of claim 19, wherein said detecting comprises measuring a fluorescent emission of said donor fluorophores.

Rule 1.126

27. The method of claim 19, wherein said at least one of said pairs is selected from the group of donor and quencher molecules consisting of fluorescein/Rhodamine X, Rhodamine X/Cy5, or fluorescein/DABCYL.

28. A method for detecting a target nucleic acid, comprising:

a. (i) hybridizing a primer P_1 to said target nucleic acid and (ii) extending P_1 by use of a polymerase to form a Strand 1, wherein the primer P_1 comprises an endonuclease recognition site at a 5'

Rule 1.26

portion of said primer P_1 that does not hybridize to the target nucleic acid;

b. (i) hybridizing a bumper B_1 to said target nucleic acid upstream from said primer P_1 and (ii) extending the bumper B_1 and removing Strand 1 from said target nucleic acid;

c. hybridizing an adaptor to Strand 1 and a primer P_2 to Strand 1, wherein the primer P_2 hybridizes upstream of the adapter;

d. (i) extending the adapter to form a Strand 2, and (ii) extending the primer P_2 to remove Strand 2 from Strand 1;

e. (i) hybridizing the primer P_1 to Strand 2 and (ii) extending the primer P_1 to form a primer P_1-extended strand;

Rule 1.26

f. (i) nicking the primer P_1-extended strand at the endonuclease recognition site incorporated into the primer P_1-extended strand and (ii) extending from the nick site to form a Strand 3 and to bump the primer P_1-extended strand that is downstream of the nick site;

g. hybridizing Strand 3 to a portion of an oligonucleotide, wherein the oligonucleotide comprises multiple pairs of donor fluorophores and quenchers, wherein the donor fluorophore and the quencher in each said pair are separated by a site that is cleavable when said cleavage site is double-stranded;

Rule 1.126

h. (i) extending Strand 3 to make the cleavage sites double-stranded and (ii) cleaving at least one of the cleavage sites; and

i. detecting said donor fluorophores,

wherein a detectable change in a fluorescence parameter of said fluorophores is indicative of the presence of said target nucleic acid.

29. A kit, comprising a single-stranded first detector oligonucleotide, comprising at least two pairs of a donor fluorophore and a quencher molecule in close proximity, wherein said donor fluorophore and said quencher molecule in each said pair are separated by a cleavage site that is cleavable in a double-stranded form, and wherein the cleavage site is double-stranded when the first detector oligonucleotide forms a duplex with a second oligonucleotide capable of being formed in the presence of a target nucleic acid.

Rule 1.126

30. The kit of claim 29, further comprising an adapter oligonucleotide that comprises a first portion, which is capable of forming a duplex with the complement of a target oligonucleotide, and a second portion, the complement of which is capable of forming a duplex with the first portion of said detector oligonucleotide.